Carbon Storage in Coarse and Fine Clay Fractions of Illitic Soils

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ABSTRACT

The storage of organic C in coarse (0.2–2 μm) and fine (<0.2 μm) clay fractions of illitic topsoils from loess was investigated in terms of the effect of particle size and mineral phase properties. We compared (i) C functional groups by ^{13}C nuclear magnetic resonance spectroscopy (NMR), (ii) the stable C isotope ratio (δ^{13}C ratio) of organic pools, and (iii) residence time of C by ^{14}C analyses. To investigate relationships between C storage and the size of mineral surface area or the amount of hydrous oxides, specific surface areas (SSAs, BET-N_2 method) and the content of dithionite-extractable Fe (Fe_d) were analyzed. The chemistry of the organic matter stored in clay subfractions was different. Compared with coarse clay, fine clay contained relatively (i) more ketonic/aldehyde, carboxyl and phenolic C, and (ii) less anomeric, O-alkyl, and methoxyl/N-alkyl C, and had (iii) a lower C content and C/N ratio and (iv) a higher δ^{13}C ratio. In 11 out of 14 fractions, C had turnover times of few centuries or less. In fine clay, the increase in SSA resulting from oxidation of organic matter explained 66% of the variation in C content, in coarse clay 97%. We calculated loadings of mineral surface area with C and Fe_d. Carbon loading exceeded Fe_d loading in coarse clay while it was of the same range in fine clay. The results may be interpreted as an indication that a certain portion of the mineral surface area controls the C content in both clay subfractions. The character of the important surface may differ between the subfractions.

O RGANIC MATTER in soil associates partly with minerals. This renders soil organic matter less susceptible to biodegradation than when it is free. Therefore, these associations are believed to be a controlling factor of C storage in soil (Baldock and Skjemstad, 2000).

Separation of soil into fractions of differently sized organo–mineral associations shows that the organic matter in the size fractions differs in chemical composition and function (Christensen, 1996). Studies have shown that C and N in whole clay fractions (<2 μm) represent predominately refractory pools with long turnover time (Balesdent and Mariotti, 1996). Nevertheless, the structural characteristics of organic matter in whole clay fractions as revealed by ^{13}C NMR spectroscopy varies between different soil types (Kögel-Knabner, 1997).

Further size separation in coarse (0.2–2 μm) and fine (<0.2 μm) clay fractions may give a better insight into the C storage than investigations on the whole clay fractions. Several studies showed that organic matter associated with fine clay fractions is more aliphatic, has relatively lower C/N ratios, relatively higher δ^{13}C ratios

and younger radiocarbon ages compared with organic matter associated with coarse clay fractions (Anderson and Paul, 1984; Baldock et al., 1992; Amelung et al., 1999). However, the cause of these differences is poorly understood. Laird et al. (2001) suggested that differences in clay mineralogy influence the humification process in clay subfractions or that humic substances with different properties are selectively adsorbed on different clay mineral species. Hydrous Al and Fe oxides, abundant constituents of the clay-size fractions, have as well been suggested as important factors for C stabilization in soils (Kaiser and Guggenberger, 2000).

In many soils, most of the C is stored in the clay-size fraction (Christensen, 1996) and consequently the concept of an inverse functional relationship between particle size and C storage capacity was developed. Baldock and Skjemstad (2000) describe the stabilization of organic materials by the soil matrix as a function of, among other factors, the chemical nature of the mineral fraction and its surfaces capable of adsorbing organic materials. However, the coarse clay fraction frequently contains a higher C content than the fine clay fraction (Anderson et al., 1981; Tiessen and Stewart, 1983; Baldock et al., 1992; Laird et al., 2001), in spite of increasing SSA with decreasing particle size. To date, the influence of mineral phase properties on C content and organic matter composition in clay subfractions remains unknown.

We investigated the effect of particle size and mineral phase properties on storage of organic C in clay subfractions of illitic surface soils. On coarse and fine clay fractions, we compared (i) the C functional groups by ^{13}C NMR spectroscopy, (ii) the quantity and δ^{13}C ratio of organic pools, and (iii) the residence time of organic C by ^{14}C analyses. We investigated relationships between C storage and the size of the mineral surface area or the amount of hydrous oxides by analyzing (i) the external SSA using untreated clay fractions and clay fractions after the removal of organic matter and (ii) the content of Fe_d.

MATERIALS AND METHODS

Soils

Samples of the topsoil from seven sites, developed from loess and under the same contemporary climate were examined. The soils represent a sequence of progressive pedogenesis from a Typic Haplustoll to Typic Haplustalfs and show varying C contents in the surface soil (Table 1). All sites have been farmed since medieval times, with a record of continuous management for at least the last 100 yr. Soil 1 has been under

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Table 1. Chemical and physical properties of the soils (Kahle et al., 2002).

Soil No.	Classification	Horizon	Depth	Inorganic C†	Organic C†	Total N†	C/N	pH (CaCl₂)	Texture‡ Sand	Silt	Clay	Fe_d†	CEC§ (pH 7)	SSA§
			cm	——————— g kg⁻¹ ———————					———— % ————			g kg⁻¹	cmol_c kg⁻¹	m² g⁻¹
1	Typic Haplustoll	Ap	0–46	0.3	19.7	1.8	11	7.3	5.9	72.9	21.2	7.0	26.7	11.1
2	Typic Haplustoll	Ap	0–36	0.0	11.6	1.2	9	6.4	3.6	74.3	22.1	7.8	17.7	15.3
3	Typic Haplustoll	Ap	0–26	0.0	15.3	1.8	9	6.2	2.9	76.0	21.1	7.4	20.3	11.2
4	Typic Argiustoll	AEp	0–35	0.0	13.7	1.4	9	6.5	1.5	78.6	19.9	6.7	17.7	12.3
5	Typic Argiustoll	Ap	0–31	0.2	12.0	1.3	9	7.1	5.4	77.2	17.5	6.4	15.2	9.2
6	Typic Haplustalf	AEp	0–33	<0.1	8.7	1.0	9	6.9	4.1	80.8	15.0	5.3	11.5	7.0
7	Typic Haplustalf	AEp	0–27	<0.1	11.3	1.2	9	6.5	8.3	76.8	14.9	5.4	11.7	5.4

† Based on 105°C dried samples.
‡ Based on particle-size distribution analyses, 105°C dried, C-free samples.
§ Based on <50-μm ground, freeze-dried samples.

grass fallow for the last 30 yr and Soil 3 was a grass fallow for 20 yr until 1991. In all soils, the clay mineral assemblage was dominated by illite (>80%) with accompanying interstratified illite, kaolinite, vermiculite, chlorite, and quartz in varying concentrations and negligible proportions of expandable 2:1 layer silicates (Kahle et al., 2002). Table 1 lists the main soil properties.

Clay Fraction Separation

The bulk soil samples were air dried, and roots and visible plant remains were removed where possible. Aggregates were crushed by hand, and the fraction >2 mm was removed by dry sieving.

The clay fraction separation was performed according to Amelung et al. (1999). Each soil sample was dispersed ultrasonically at 60 J mL⁻¹ in a suspension of one part soil to five parts water by weight. The output of ultrasonic energy was determined by measurement of heat production. The coarse sand fraction (>200 μm) was removed by wet sieving. The remaining suspension was diluted to a soil/water ratio of 1:10 and dispersed with ultrasound at 440 J mL⁻¹. Repeated centrifugation (usually 15 centrifugation runs) was used to separate first the fine clay (<0.2 μm) and thereafter the coarse clay fraction (0.2–2 μm). The clay fractions were flocculated with MgCl₂, dialyzed against deionized water and freeze-dried. Clay fraction separation was repeated two times per sample to control the amount of the clay fractions separated. The average recovery rate varied between 98 and 101% of the original material.

A part of each clay-size fraction was treated several times with hydrogen peroxide in a water bath at 70°C to remove organic matter. After the peroxide treatment, the clay fractions were washed once with deionized water and freeze-dried. Wet oxidation removed more than 93% of the organic matter in all samples, and we designate these samples as C-free.

Chemical Characterization

Total C and N were determined by dry combustion of ground, 105°C dried subsamples with a CNS analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany). Since only traces of inorganic C were found in the bulk soils, we regard the total C of the clay fractions as organic.

The δ¹³C ratios of ground, 105°C dried clay fractions were obtained with an isotope ratio mass spectrometer (Finnigan MAT Delta Plus, Thermo Finnigan GmbH, Bremen, Germany) coupled with an elemental analyzer (Fisons EA 1108, Thermo Finnigan GmbH, Bremen, Germany). The isotope ratios of the samples were compared with the isotope ratio of acetanilide, calibrated against an IAEA-standard (CO-8).

We determined the content of Fe_d according to Blakemore et al. (1987) by shaking 0.25 g of freeze-dried clay fraction with 1 g of sodium dithionite and 50 mL of sodium citrate (22%) for 16 h. Ten milliliters of 0.05 M MgSO₄ was added as a flocculant, the sample was centrifuged, and the supernatant decanted. The supernatant was made up to 100 mL with double-deionized water, and Fe concentration in it was measured by inductively coupled plasma spectroscopy.

The SSA was determined by adsorption of N₂ at 77 K and subsequent desorption of the N₂ with a Quantachrome Monosorb 1-point BET-instrument (Quantachrome Instruments, Boynton Beach, FL) on freeze-dried clay fractions (untreated and C-free).

Carbon-13 Cross Polarization Magic-Angle Spinning NMR Spectroscopy

Solid-state ¹³C NMR spectra were obtained on a Bruker DSX 200 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) operating at a ¹³C resonance frequency of 50.3 MHz by using the cross polarization magic-angle spinning (CP-MAS) technique. Samples were packed into a cylindrical zirconia rotor (diameter 7 mm) with Kel-F caps, and spun at a frequency of 6.8 kHz. A contact time of 1 ms and a pulse delay of 200 to 400 ms were used. A ramped ¹H-pulse was used during contact time to circumvent inexact Hartmann-Hahn conditions. After accumulation of $133 - 822 \times 10^3$ scans and before Fourier transformation, a line broadening of 100 to 250 Hz was applied. The ¹³C chemical shifts were referenced to tetramethylsilane (=0 ppm) and calibrated with glycine. The spectra were divided into eight chemical shift regions (Knicker and Lüdemann, 1995): ketonic/aldehyde C (220–185 ppm), carboxyl C (185–160 ppm), phenolic C (160–140 ppm), sp² C (140–110 ppm), anomeric C (110–90 ppm), O-alkyl C (90–60 ppm), methoxly/N-alkyl C (60–45 ppm), and alkyl C [45–(−10) ppm]. Relative C distribution for the regions was determined via an integration routine supplied with the instrument software.

Carbon-14 Analysis and Calculation of Turnover Times

Radiocarbon content of organic matter was measured by accelerator mass spectrometry at the Lawrence Livermore National Laboratory on targets prepared by H reduction (Vogel et al., 1984). Before target preparation, clay fractions were held under an atmosphere of HCl overnight to avoid possible contamination with inorganic C and were subsequently dried at 105°C. Radiocarbon content is expressed as Δ¹⁴C, the deviation of the ¹⁴C/¹²C ratio in the sample from that of an oxalic standard, with decay and ¹³C corrections according to Stuiver and Polach (1977). In the late 1950s to early 1960s, aboveground nuclear weapons testing caused a large increase in atmospheric ¹⁴CO₂. The Δ¹⁴C unit is normalized such that

the pre-1950 atmosphere was roughly $\Delta^{14}C = 0$. A negative $\Delta^{14}C$ indicates radioactive decay of older material has occurred and positive values of $\Delta^{14}C$ indicate the presence of bomb-produced ^{14}C.

We used a one-pool, time-dependent, steady-state model to estimate turnover from our radiocarbon content. Radiocarbon content of soil organic matter was modeled as

$$F_C(t) = (1/T) \times F_{atm}(t) + F_C(t - 1)$$
$$\times [1 - (1/T) - \lambda_{14C}] \qquad [1]$$

where F_C is fraction modern of soil C, F_{atm} is fraction modern of atmosphere, T is the turnover time (reciprocal of decay constant, k), t is the point of time, and λ_{14C} is the rate constant for radioactive decay of ^{14}C ($\lambda_{14C} = 1.245 \times 10^{-4}$ yr^{-1}). $\Delta^{14}C$ is calculated as

$$\Delta^{14}C = (F_C - 1) \times 1000. \qquad [2]$$

The modeling started in Year 1900, with

$$F_C = (1/T)/[(1/T) + \lambda_{14C}], \qquad [3]$$

the fraction modern of natural radiocarbon at steady state. Turnover time was determined by an iterative procedure.

Statistics

Statistical regression analyses were conducted using SPSS for Windows (version 9.0, SPSS Inc., Chicago, IL). Significance of the differences between properties of the coarse and fine clay fractions was tested by means of a paired t test.

RESULTS AND DISCUSSION
Properties of the Clay Fractions

Table 2 lists the properties of the fine and coarse clay fractions. The mass proportions of the clay fractions obtained with particle-size fractionation were higher than those obtained with particle-size distribution analysis (Table 1). This indicates an adequate dispersion of whole soil by the ultrasonic energy applied. In all soils the contribution of coarse clay to whole clay fraction was larger than the contribution of fine clay.

The C content of the coarse clay fractions was significantly higher than the C content of the fine clays (Table 2). No significant difference was observed for the N content of the two clay subfractions therefore the C/N ratio of the coarse clay was significantly higher

than that of the fine clay. This suggests that the organic matter associated with fine clay is enriched in N compared with the organic matter associated with coarse clay. Similar results have been observed in several studies of various soils (Anderson et al., 1981; Tiessen and Stewart, 1983; Baldock et al., 1992; Laird et al., 2001).

Chemical Structure of Organic Matter and Turnover Times

Carbon-13 NMR spectra of the coarse and fine clay fractions are depicted in Fig. 1, relative signal intensities are presented in Table 3. For soils across the pedogenic gradient, the spectra within a particle-size fraction demonstrated somewhat similar distributions of signal intensities. However, between the clay subfractions a shift in signal distributions was observed.

The signal at 30 ppm may originate from methylenic C in long-chain aliphatic compounds of varying origin (Kögel-Knabner, 1997). Resonances between 20 and 30 ppm, which were dominant in the spectra of the clay fractions, indicate the presence of rather short chain aliphatic material as compared with a signal at 30 ppm (Baldock et al., 1992). The signals around 72 and 105 ppm, together with shoulders around 65 and 80 to 90 ppm, are tentatively assigned to polysaccharides (Kögel-Knabner, 1997). However, signals of ether and alcohols may also contribute to these signals. The resonances around 175 ppm are derived from carboxylic, amide, or ester C in various compounds (Baldock et al., 1992). The aromatic region (110–160 ppm) was characterized by broad signals around 130 ppm, whereas the intensity of the signals around 150 ppm, mainly assignable to phenol C and indicating the presence of lignin units, was generally low, especially in the coarse clay fractions. This reveals that lignin units, even highly altered, did not constitute a major component of the organic matter. Sp^2–hybridized C of various origin, for example C in condensed aromatic rings like in charred plant residues (Kiem et al., 2000), may contribute to the signals around 130 ppm. The peak around 56 ppm may originate from methoxyl groups in lignin, but in case of our clay fractions, which showed low C/N ratios, it is more likely that it derives from N-substituted alkyl C in amino acids (Guggenberger et al., 1996).

Table 2. Properties of the fine (<0.2 μm) and coarse (0.2–2 μm) clay fractions: content of clay subfraction in soil, contribution of clay subfraction to whole clay fraction, content of organic C and total N, C/N ratios, and specific surface area (SSA).

Clay fraction of soil	Clay fraction content†		Contribution to whole clay†		Organic C‡**		Total N‡		C/N ratio***		SSA§	
	<0.2 μm	0.2-2 μm	<0.2 μm	0.2-2 μm	<0.2 μm	0.2-2 μm	<0.2 μm	0.2-2 μm	<0.2 μm	0.2-2 μm	<0.2 μm	0.2-2 μm
	—— mass % ——		—— % ——		—————— g kg^{-1} ——————						—— m^2 g^{-1} ——	
1	7.5	16.2	32	68	38.8	64.0	4.4	5.5	9	12	63.2	24.5
2	10.8	13.1	45	55	28.3	39.1	3.9	4.1	7	10	72.1	30.1
3	9.1	16.2	36	64	34.5	47.8	4.5	5.1	8	9	57.7	20.4
4	9.2	11.5	45	55	29.0	45.1	4.1	5.1	7	9	73.4	26.2
5	7.8	12.3	39	61	29.5	44.0	4.3	5.2	7	8	63.2	21.2
6	5.1	10.9	32	68	29.2	32.6	4.6	4.2	6	8	66.0	24.3
7	5.3	11.5	32	68	34.3	40.2	4.7	4.5	7	9	50.0	21.4

** Significant difference between coarse and fine clay fraction at the 0.01 probability level.
*** Significant difference between coarse and fine clay fraction at the 0.001 probability level.
† Based on particle-size fractionation.
‡ Based on 105°C dried samples.
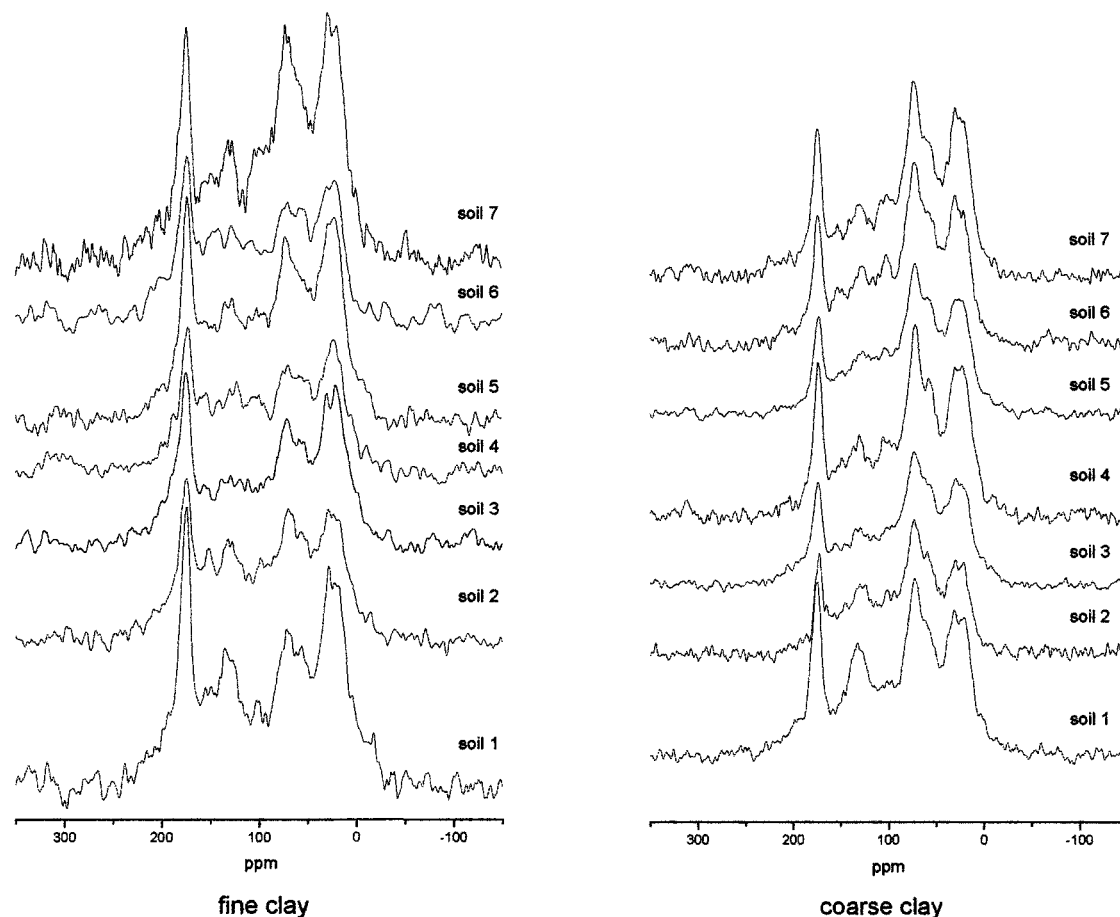§ Based on freeze-dried samples.

Fig. 1. Carbon-13 nuclear magnetic resonance spectra of the fine (<0.2μm) and the coarse (0.2–2 μm) clay fractions.

Significant differences in the relative intensity distributions between the spectra of the clay subfractions were observed for several chemical shift regions (Table 3). Compared with the coarse clay fraction, the fine clay had relatively more ketonic/aldehyde C, carboxyl C and phenolic C and relatively less anomeric C, O-alkyl C, and methoxly/N-alkyl C. For sp^2 C and alkyl C no trends existed. Therefore, the organic matter in fine clay fractions seems to contain relatively more carboxylic, amide or ester C, and relatively less carbohydrates, alcohols, or ethers than the organic matter associated with the coarse clay fractions, independent of pedogenic stage of the soils. However, this result may have been influenced by a number of limitations associated with solid-state ^{13}C CPMAS NMR spectroscopy including the existence of paramagnetic species leading to a loss of signal intensity for close C nuclei.

The alkyl/(O, N-alkyl) ratio in the fine clay fractions was significantly higher compared with the coarse clay (Table 3). Baldock et al. (1997) suggested the use of the ratio of alkyl C (45 to −10 ppm) to O, N-alkyl C (110–45 ppm) as an indicator for the degree of decomposition of organic matter in soils. Baldock et al. (1992) fractionated two Mollisols and two Oxisols and concluded from NMR spectra that the O, N-alkyl C content decreases and the alkyl C content increases with decreasing particle size. They deduced that the mineralization process accounts for the decrease in the amount of

O, N-alkyl C, and that the accumulation of alkyl C in finer fractions results from both a selective preservation and an in situ synthesis. According to this interpretation of the alkyl/(O, N-alkyl) ratio, the organic matter in the fine clay fractions would be more decomposed than the organic matter in the coarse clay. However, it is questionable if this conclusion can be drawn for clay subfractions. First, there is evidence in literature that in clay fractions, the carbohydrates that cause signals in the O-alkyl region are mainly in situ synthesized microbial metabolites and not sugars derived from plant decomposition (Guggenberger et al., 1995). Second, we did not observe a significant increase in alkyl-C content from coarse to fine clay. Combined, this suggests that differences in the composition of organic matter associated with clay subfractions may not be caused by a progression of decomposition from coarse to fine.

Another explanation for the differences in the composition of organic matter may be the association of smaller organic compounds to fine clay compared with coarse clay combined with differences in the chemical composition of the organic matter of different size. Soil organic matter, especially microbial metabolites, comprises a wide range of sizes and chemical compositions that are available for associations with clay minerals. Selective associations of organic substances to silicate clay minerals or oxides are imaginable as well. In this case, differences in silicate clay mineralogy or oxides

Table 3. Relative contributions of chemical shift regions to the total signal intensity in ^{13}C NMR spectroscopy of fine (<0.2 μm) and coarse (0.2–2 μm) clay fractions.

Clay fraction of soil	Ketonic/Aldehyde C*** 220–185 ppm		Carboxyl C*** 185–160 ppm		Phenolic C** 160–140 ppm		sp² C 140–110 ppm		Anomeric C* 110–90 ppm		O-Alkyl C*** 90–60 ppm		Methoxyl/N-Alkyl C*** 60–45 ppm		Alkyl C 45–(−10) ppm		Alkyl C† O, N–Alkyl C	
	<0.2 μm	0.2–2 μm	<0.2 μm	0.2–2 μm	<0.2 μm	0.2–2 μm	<0.2 μm	0.2–2 μm	<0.2 μm	0.2–2 μm	<0.2 μm	0.2–2 μm	<0.2 μm	0.2–2 μm	<0.2 μm	0.2–2 μm	<0.2 μm	0.2–2 μm
									%									
1	7.5	5.4	16.1	13.2	7.0	5.5	13.1	14.5	5.7	7.1	14.6	20.9	7.2	8.4	28.7	25.0	1.0	0.7
2	6.7	2.2	14.6	10.6	7.8	6.5	13.9	14.7	7.3	8.8	16.8	24.5	7.8	9.6	25.0	23.1	0.8	0.5
3	7.7	5.0	15.0	11.3	5.5	5.4	10.9	11.5	6.2	7.9	16.6	23.3	7.8	9.5	30.3	26.0	1.0	0.6
4	5.6	3.1	14.5	11.9	7.8	5.5	14.1	11.3	8.2	7.9	15.8	23.1	8.1	9.6	25.9	27.6	0.8	0.7
5	4.7	2.4	14.0	10.0	7.1	5.2	13.3	12.4	8.0	8.3	17.7	24.3	8.0	10.4	27.1	27.0	0.8	0.6
6	6.7	3.7	15.3	10.6	8.2	5.5	13.8	12.1	7.4	8.4	16.1	23.9	8.2	10.0	24.3	25.8	0.8	0.6
7	5.7	4.1	12.5	11.4	5.6	4.6	10.3	10.5	7.5	8.0	19.8	23.6	8.5	9.5	30.0	28.4	0.8	0.7

* Significant difference between coarse and fine clay fraction at the 0.05 probability level.
** Significant difference between coarse and fine clay fraction at the 0.01 probability level.
*** Significant difference between coarse and fine clay fraction at the 0.001 probability level.
† Relative contribution 45 to −10 ppm divided by relative contribution 110–45 ppm.

between coarse and fine clay would create differences in the chemical composition of the organic matter associated. Laird et al. (2001) observed a dominance of the alkyl peak in the NMR spectra of the coarse clay fraction from a Webster pedon and this dominance increased with decreasing particle size of the clay subfractions. Since clay mineralogy of these subfractions differed, the authors inferred a relationship between clay mineralogy and the chemical nature of the associated humic substances.

The organic matter in the clay subfractions had turnover times of a few centuries or less, except Soil 1 and 2 with turnover times of more than a 1000 yr (Table 4). These are relatively fast turnover times compared with turnover times of stable organic pools in the range of several hundreds to several thousands of years reported in other studies (Hsieh, 1993; Torn et al., 1997). Although we modeled turnover time by treating the C in a clay fraction as a single homogeneous pool, it may be a combination of long residence time material and a significant portion of organic matter with faster turnover times. Since the existence of organic matter not intimately associated with the clay minerals cannot be excluded, such organic matter may have had an influence on the results. Free organic matter could have very fast or very long turnover times.

For the coarse clay fractions a significant and positive relationship between turnover time and sp² C content of organic matter was observed (Table 5). Together with methoxyl/N-alkyl C content, which had a negative impact, sp² C content explained 99% of the variation in turnover time. This may indicate that in the coarse clay fractions with longer turnover times a higher portion of condensed aromatic rings is present compared with coarse clay fractions with faster turnover times. Charred organic materials could be the reason for the higher portion of condensed aromatic rings. For the fine clay fraction, no relationships were observed.

The pairwise comparison of the two clay sizes elucidated that in most pairs the coarse fraction had slower turnover times than the fine fraction but the differences between the modeled turnover times of the fractions from Site 5 to 7 are not significant given the reported analytical errors of Δ^{14}C determination. Anderson and Paul (1984) found the oldest C of a soil in the coarse clay fraction and much younger C in the fine clay. They concluded that the organic C of the latter was considerably more active than that associated with the coarse clay. In two other soils, they observed ^{14}C ages of 200 yr to modern for the organic C of the clay-size fractions. Scharpenseel et al. (1986) examined particle-size fractions of three Mollisols and observed a maximum of the ^{14}C age in coarse clay fractions. They used sodium pyrophosphate to disperse the soils and suggested that fine clay fractions were contaminated by young humic material that was dissolved by pyrophosphate. Unfortunately, the effect of procedures established for particle-size fractionation on C chemistry and C isotopic content is poorly understood.

The stable C isotope ratio (δ^{13}C) increased significantly from coarse to fine clay fraction (Table 4). For

Table 4. Natural abundance ^{13}C (δ^{13}C) ratios, Δ^{14}C values, and modeled turnover times of organic matter in fine (<0.2 µm) and coarse (0.2–2 µm) clay fractions.

Clay fraction of soil	δ^{13}C***		Δ^{14}C		Turnover time	
	<0.2 µm	0.2–2 µm	<0.2 µm	0.2–2 µm	<0.2 µm	0.2–2 µm
	‰				yr	
1	−24.6	−25.2	−8.1	−172.6	352	1761
2	−24.6	−25.1	−140.6	−129.0	1412	1293
3	−24.6	−25.3	36.5	−18.1	188	403
4	−24.2	−24.9	108.5	53.2	79	151
5	−24.7	−25.4	4.0	−0.8	296	317
6	−24.5	−25.0	19.9	25.4	237	219
7	−25.2	−25.7	68.3	65.1	125	130

*** Significant difference between coarse and fine clay fraction at the 0.001 probability level.

fractions from native pasture soils, Amelung et al. (1999) interpreted the increase in δ^{13}C ratio with decreasing particle size to indicate that the organic matter of the finer fractions was increasingly altered by microbes. This conclusion is questionable for the clay subfractions as discussed above. Balesdent and Mariotti (1996) suggested that the difference in δ^{13}C ratio is correlated with the age of the C and one potential reason for that would be the change in δ^{13}C of atmospheric CO_2 during historic time. In the case of the clay fractions, there was no clear trend for the turnover times of organic matter, therefore age of C solely seems not to be able to explain differences in δ^{13}C ratios. However, we determined differences in the chemical composition of the organic matter in coarse and fine clay fractions. Since C functional groups can differ in δ^{13}C ratio (Boutton, 1996), this may have caused the difference in δ^{13}C ratio of the clay subfractions.

Relations between Surface Properties, Iron Oxide Content, and Carbon Storage

Removing the organic matter led to distinct increases in the specific surface area of the clay subfractions [SSA, Table 2, and SSA(c), Table 6]. This increase (ΔSSA, Table 6) was significantly higher in fine clay than in coarse clay fractions. An increase in the BET-N_2 surface area of soils and clay-sized fractions after removal of C was observed before (Christensen, 1996). The phenomenon is commonly attributed to the coverage of mineral surfaces with organic matter, which itself seems to have only a negligible N_2 surface area (Theng et al., 1999). The removal of organic matter renders mineral surfaces accessible to N_2 gas. An additional reason may be the dispersion of <2-µm microaggregates through the destruction of organic matter (Feller et al., 1992).

The SSA(c) and the C content of the untreated fraction were significantly and positively related in coarse clay (Table 5). No relationship was found for fine clay fractions. The relationship between C content of the untreated fraction and ΔSSA was positive and significant for both clay subfractions. In the fine clay, ΔSSA explained 66% of the variation in C content, in the coarse clay, 97% of the variation was explained (Table 5). Keil et al. (1994) observed a strong positive relationship between organic C and SSA(c) for five coastal marine sediments and their hydrodynamic fractions, including coarse and fine clay fractions, using similar methods to our study. In contrast, we observed two relationships with different slopes for the two clay sub-

fractions and ΔSSA explained a larger part of the variation in C content than SSA(c).

Our results may be interpreted as an indication that the size of a specific portion of the mineral surface area controls the C storage. In coarse clay fractions, the removal of organic matter may have released approximately the surface area that is covered by organic matter. In the fine clay fractions, the relationship between ΔSSA and C content was weaker. The removal of organic matter in fine clay may not only have uncovered surface area covered by organic matter but may have made additional surface area accessible as a result of the dispersion of microaggregates or of the increased accessibility of micropores. Further, organic matter not intimately associated with the clay minerals may exert an influence on the relationships. Additionally, variations in the C input to the clay fractions may have influenced the extent of the C cover of the surface areas in the samples. However, since the sites chosen were characterized by a virtually identical contemporary climate and vegetation/land-use history, we think that this option did not introduce a significant variability in the C storage.

The content of Fe_d, an indicator for the content of Fe oxides, was significantly higher in the fine clay fraction

Table 5. Single and multiple regression analyses ($n = 7$), Fe_d = Fe_d content of untreated fraction, SSA(c) = specific surface area of C-free fraction, ΔSSA = increase in specific surface area after removal of organic matter, sp^2 C and methoxyl/ N-alkyl C = relative contribution of C functional groups measured by ^{13}C NMR spectroscopy.

Regression equations and R^2 values

Coarse clay

Turnover time (yr) = −3952.4 + 366.9 sp^2 C (%)	$R^2 = 0.85$**
Turnover time (yr) = 980.1 + 299.8 sp^2 C (%) − 429.7 methoxyl/N-alkyl C (%)†	$R^2 = 0.99$***
C content (g kg^{-1}) = −3.76 + 0.77 SSA(c) (m^2 g^{-1})†	$R^2 = 0.85$**
C content (g kg^{-1}) = 9.81 + 1.03 ΔSSA (m^2 g^{-1})	$R^2 = 0.97$***
C content (g kg^{-1}) = 52.8 + 0.77 SSS(c) (m^2 g^{-1}) − 2.64 Fe_d (g kg^{-1})	$R^2 = 0.98$***

Fine clay

C content (g kg^{-1}) = 2.86 + 0.48 ΔSSA (m^2 g^{-1})†	$R^2 = 0.66$*
C/N ratio = 18.21− 0.415 Fe_d (g kg^{-1})	$R^2 = 0.67$*
Methoxyl/N-alkyl C (%) = 1.75 + 0.24 Fe_d (g kg^{-1})†	$R^2 = 0.84$**

* Significant difference between coarse and fine clay fraction at the 0.05 probability level.
** Significant difference between coarse and fine clay fraction at the 0.01 probability level.
*** Significant difference between coarse and fine clay fraction at the 0.001 probability level.
† Constant not significant.

Table 6. Dithionate-extractable Fe content of untreated fine (<0.2 μm) and coarse (0.2–2 μm) clay fractions, specific surface area of C-free clay fractions [SSA(c)], increase in SSA after removal of organic matter (ΔSSA), C and Fe_d loadings of the surface area.

Clay fraction of soil	Fe_d†***		SSA(c)†***		ΔSSA‡***		Carbon loading§		Fe_d loading¶	
	<0.2 μm	0.2-2 μm	<0.2 μm	0.2-2 μm	<0.2 μm	0.2-2 μm	<0.2 μm	0.2-2 μm	<0.2 μm	0.2-2 μm
	——— g kg⁻¹ ———		——————— m² g⁻¹ ———————				——— mg C m⁻² ———		——— mg Fe_d m⁻² ———	
1	23.6	21.1	141.3	85.1	68.6	51.2	0.29	0.84	0.18	0.28
2	26.4	23.7	134.1	64.7	55.4	30.2	0.22	0.65	0.21	0.39
3	25.1	19.7	135.2	62.4	69.5	36.8	0.27	0.83	0.20	0.34
4	25.9	21.9	138.2	65.1	57.9	33.9	0.22	0.75	0.20	0.36
5	26.2	20.5	131.2	61.3	61.3	35.5	0.24	0.78	0.21	0.36
6	28.0	22.3	123.1	48.8	50.9	21.7	0.25	0.71	0.24	0.48
7	28.0	20.5	117.9	51.7	60.9	26.7	0.31	0.84	0.25	0.43

*** Significant difference between coarse and fine clay fraction at the 0.001 probability level.
† Based on freeze-dried samples.
‡ Difference between SSA of untreated clay fraction (Table 2) and unit-corrected SSA of C-free clay fraction, correction: SSA(c)$_{corr.}$ = SSA(c) ×
　(1− {[1.72 × organic C (g kg⁻¹)]/1000}), organic C is the content of organic C of the untreated clay fraction.
§ Carbon loading: quotient of content of organic C of untreated clay fraction and SSA(c)$_{corr.}$.
¶ Dithionate-extractable Fe loading: quotient of Fe_d content of untreated clay fraction and SSA(c)$_{corr.}$.

compared with the coarse clay (Table 6). The relationships between Fe_d content and C content were not significant, but a negative trend over all fractions was observed. In coarse clay fractions, the Fe_d content together with the SSA(c) explained 98% of the variation in C content but Fe_d content had a negative impact (Table 5). The result may either indicate that there is no specific control of C storage in coarse clay through Fe oxides or that the variable Fe_d content is of limited predictive power.

We calculated the loading of mineral surface area with C and Fe_d by dividing C content or Fe_d content of the untreated fraction by the SSA(c) (Table 6). In all fine clay fractions, C and Fe_d loadings were in the same range, while in the coarse clay C loadings exceeded Fe_d loadings. Both the C loadings and the Fe_d loadings were higher in coarse clay compared with fine clay.

The results may be interpreted as an indication that the character of the mineral surface associated with the organic matter differs between the coarse and the fine clay fraction. The same range of C and Fe_d loadings in all fine clay fractions may indicate that Fe oxides predominantly provide the important surface area for the association with organic matter in fine clay. In the coarse clay fraction, silicate mineral surfaces may be more important for C storage than Fe oxides. This may be indicated by the C loading exceeding the Fe_d loading and by the negative impact of Fe_d content on the variation in C content as mentioned above.

If different mineral surfaces are actually important in the clay subfractions, a selective association of organic matter by oxides and silicate minerals may have caused the differences in the chemistry of the organic matter associated. However, we only observed a significant and negative relationship between the Fe_d content and the C/N ratio and a significant and positive relationship between the Fe_d content and the content of methoxyl/N-alkyl C within the fine clay fractions (Table 5). This may indicate that Fe oxides in fine clay specifically associate with N-rich organic matter.

CONCLUSIONS

Our results may be interpreted as an indication that in fine and coarse clay fractions of illitic topsoils the size of a specific portion of the mineral surface area controls the C content. While in the fine clay fraction Fe oxides may provide the important surface area, silicate mineral surface areas may be more important for C storage in the coarse clay fraction.

Carbon storage in the clay subfractions differed in the chemistry of the organic matter stored but there was no significant difference in the turnover time of C associated with the coarse versus fine clay fraction. The modeled C turnover times reflected the presence of organic material with relatively fast turnover associated with both clay subfractions. Compared with coarse clay, the fine clay fraction generally contained (i) relatively more carboxylic, amide, or ester C; and (ii) relatively less carbohydrates, alcohols, or ethers; had (iii) a lower C/N ratio; and (iv) a higher $\delta^{13}C$ ratio. While the differences in the composition of organic matter in the clay subfractions may be caused by a progressive decomposition of organic matter from coarse to fine clay, we suggest two alternative explanations: (i) the association of smaller organic materials to fine clay compared with coarse clay combined with differences in chemical composition of organic matter of different size or (ii) selective association of organic substances to Fe oxides versus silicate clay minerals combined with a possibly greater importance of oxides for C storage in fine clay fractions versus coarse clay fractions.

REFERENCES

Amelung, W., R. Bol, and C. Friedrich. 1999. Natural ^{13}C abundance: A tool to trace the incorporation of dung-derived carbon into soil particle-size fractions. Rapid Commun. Mass Spectrom. 13: 1291–1294.

Anderson, D.W., and E.A. Paul. 1984. Organo-mineral complexes and their study by radiocarbon dating. Soil Sci. Soc. Am. J. 48:298–301.

Anderson, D.W., S. Saggar, J.R. Bettany, and J.W.B. Stewart. 1981.

Particle size fractions and their use in studies of soil organic matter: I. The nature and distribution of forms of carbon, nitrogen, and sulfur. Soil Sci. Soc. Am. J. 45:767–772.

Baldock, J.A., and J.O. Skjemstad. 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Org. Geochem. 31:697–710.

Baldock, J.A., J.M. Oades, A.G. Waters, X. Peng, A.M. Vassallo, and M.A. Wilson. 1992. Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR spectroscopy. Biogeochemistry 16:1–42.

Baldock, J.A., J.M. Oades, P.N. Nelson, T.M. Skene, A. Golchin, and P. Clarke. 1997. Assessing the extent of decomposition of natural organic materials using solid-state ^{13}C NMR spectroscopy. Aust. J. Soil Res. 35:1061–1083.

Balesdent, J., and A. Mariotti. 1996. Measurement of soil organic matter turnover using ^{13}C natural abundance. p. 83–111. *In* T.W. Boutton and S. Yamasak (ed.) Mass Spectrometry of Soils. Marcel Dekker, Inc., New York.

Blakemore, L.C., P.L. Searle, and B.K. Daly. 1987. Methods for chemical analysis of soils. New Zealand Soil Bureau, Scientific Rep. 80. Department of Scientific and Industrial Research, Lower Hutt, New Zealand.

Boutton, T.W. 1996. Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. p. 47–82. *In* T.W. Boutton and S. Yamasak (ed.) Mass spectrometry of soils. Marcel Dekker, Inc., New York.

Christensen, B.T. 1996. Carbon in primary and secondary organomineral complexes. p. 97–165. *In* M.R. Carter and B.A. Stewart (ed.) Structure and organic matter storage in agricultural soils. Lewis Publishers, Boca Raton, FL.

Feller, C., E. Schouller, F. Thomas, J. Rouiller, and A.J. Herbillon. 1992. N_2–BET specific surface areas of some low activity clay soils and their relationships with secondary constituents and organic matter contents. Soil Sci. 153:293–299.

Guggenberger, G., W. Zech, and R.J. Thomas. 1995. Lignin and carbohydrate alteration in particle-size separates of an oxisol under tropical pastures following native savanna. Soil Biol. Biochem. 27:1629–1638.

Guggenberger, G., R.J. Thomas, and W. Zech. 1996. Soil organic matter within earthworm casts of an anecic-endogeic tropical pasture community, Colombia. Appl. Soil Ecol. 3:263–274.

Hsieh, Y.-P. 1993. Radiocarbon signatures of turnover rates in active soil organic carbon pools. Soil Sci. Soc. Am. J. 57:1020–1022.

Kahle, M., M. Kleber, and R. Jahn. 2002. Carbon storage in loess derived surface soils from Central Germany: Influence of mineral phase variables. J. Plant Nutr. Soil Sci. 165:141–149.

Kaiser, K., and G. Guggenberger. 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Org. Geochem. 31:711–725.

Keil, R.G., E. Tsamakis, C.B. Fuh, J.C. Giddings, and J.I. Hedges. 1994. Mineralogical and textural controls on the organic composition of coastal marine sediments: Hydrodynamic separation using SPLITT-fractionation. Geochim. Cosmochim. Acta 58:879–893.

Kiem, R., H. Knicker, M. Körschens, and I. Kögel-Knabner. 2000. Refractory organic carbon in C-depleted arable soils, as studied by ^{13}C NMR spectroscopy and carbohydrate analysis. Org. Geochem. 31:655–668.

Knicker, H., and H.-D. Lüdemann. 1995. N-15 and C-13 CPMAS and solution NMR studies of N-15 enriched plant material during 600 days of microbial degradation. Org. Geochem. 23:329–341.

Kögel-Knabner, I. 1997. ^{13}C and ^{15}N NMR spectroscopy as a tool in soil organic matter studies. Geoderma 80:243–270.

Laird, D.A., D.A. Martens, and W.L. Kingery. 2001. Nature of clay-humic complexes in an agricultural soil: I. Chemical, biochemical, and spectroscopic analyses. Soil Sci. Soc. Am. J. 65:1413–1418.

Scharpenseel, H.W., K. Tsutsuki, P. Becker-Heidmann, and J. Freytag. 1986. Untersuchungen zur Kohlenstoffdynamik und Bioturbation von Mollisolen. J. Plant Nutr. Soil Sci. 149:582–597.

Stuiver, M., and H.A. Polach. 1977. Reporting of ^{14}C data. Radiocarbon 19:355–363.

Theng, B.K.G., G.G. Ristori, C.A. Santi, and H.J. Percival. 1999. An improved method for determining the specific surface areas of topsoils with varied organic matter content, texture and clay mineral composition. Eur. J. Soil Sci. 50:309–316.

Tiessen, H., and J.W.B. Stewart. 1983. Particle-size fractions and their use in studies of soil organic matter: II. Cultivation effects on organic matter composition in size fractions. Soil Sci. Soc. Am. J. 47:509–514.

Torn, M.S., S.E. Trumbore, O.A. Chadwick, P.M. Vitousek, and D.M. Hendricks. 1997. Mineral control of soil organic carbon storage and turnover. Nature 389:170–173.

Vogel, J.S., J.R. Southon, D.E. Nelson, and T.A. Brown. 1984. Performance of catalytically condensed carbon for use in accelerator mass spectrometry. Nucl. Instrum. Meth. B. 233:289–293.